April 24, 1973

Dr. Ian Macpherson
Imperial Cancer Research Fund
Lincoln's Inn Fields
London, WC 2, England

Dear Dr. Macpherson,

I am sorry we have been so long in relaying information about the hamster revertant experiments, but each week it has seemed as though one more bit of data would give us a clearer idea of what to do next.

To begin with the DNA you recently sent: unfortunately, the DNA was clearly, upon arrival, not sufficiently intact to perform convincing integration studies. It was only minimally viscous at a high concentration, only 30% of the DNA entered into networks (usually 80-95% of mammalian DNA forms networks), and in alkaline sucrose gradients (see Figure 1) the sedimentation value proved to be 20-25 S in relation to $\lambda\lambda$ DNA (53S) marker. Thus, the DNA had a single stranded weight of approximately 3×10^6. Based upon our previous findings with B77 transformed 3T3 cells (see Lepetit manuscript, Fig. 10), we expected that most of the integrated RSV DNA would have been detached from reiterated sequence DNA. Thus, the network fractions would be depleted of virus-specific DNA. This is, in fact, observed in Figure 2, and the amount of RSV DNA in transformed and revertant DNA seems about the same. Thus, we have an indication that the viral DNA is integrated in both cell types and, roughly speaking, in the same pattern, since equal reduction in size of the cell DNA equally reduces the amount of virus-specific DNA covalently linked to reiterated sequences. However, it would be nice to demonstrate integration more convincingly with very high molecular weight DNA. I think we might have more luck, in view of the distance between us, if you could send 2×10^8 or more cells of each type in "DNA buffer" (0.02M Tris, pH 8, 0.01M EDTA, 0.1M NaCl) after lysis of 5×10^6-10^7 cells/ml with 1% SDS. I think air mail shipping at room temperature (do not freeze) will be all right and we'll finish the extraction here, make networks, and assay.

Things went rather more smoothly in the RNA department. As shown by the experiments in Fig. 3, there is clearly more annealing of labeled single-stranded DNA (synthesized by Schmidt-Ruppin virus polymerase) to RNA from transformed cells than to RNA from revertant cells. The amount of virus specific RNA are small - approximately 0.0001% of the cell RNA, or about 1 molecule per cell - but the results are

reproducible. A reaction with B77 transformed 3T3 cells is shown for comparison. HeLa RNA, as you can see, does not react at all. At present, this reaction has been pushed to its logistical limits: 20 mg/ml of RNA, 0.6 M Na^+, 100 hour inactivation. If it were possible to receive another shipment of RNA from these cells - approximately 2-10 times the amount in the first shipment - we could attempt to augment the reaction by running the RNA through a poly dT-cellulose column. This technique provides a fraction of RNA 3-20 fold enriched for virus-specific sequences, allowing us to carry the reactions to higher "Cot" values. In addition, we learn whether the virus-specific RNA has poly A attached in these cells. We are, in addition, pursuing several relevantpmatters in B77/3T3 cells: presence of virus-specific RNA in polysomes; nuclear versus cytoplasmic location of RSV RNA; and a competition assay between cell RNA and labeled 70S RNA which permits a precise estimate of the amount of the genome transcribed. (The unequal copying of RSV RNA into single-stranded DNA, even in the presence of actinomycin, when the entire genome is copied, albeit assymetrically, forbid equation of the extent of transcription with the percentage of single stranded DNA hybridized t cell RNA.) When these techniques are well worked out, perhaps we can pursue them in hamster cells if they seem appropriate.y

In particular, nuclear/cytoplasmic fractionation appears promising in regard to B77/3T3 cells, since nuclear RNA seems considerably enriched in RSV RNA. The possibility that RSV RNA never gets to the cytoplasm in the revertant will is a titillating one, and we would like your opinion about whether you'd want to do the cellular fraction there or to send the appropriate living cells to us.

I am tentatively expecting to be briefly in London about the middle of July. If this happens, I hope we will have a chance to discuss these matters further. I will send you explicit dates when I have them.

Again, thanking you for your efforts in this interesting venture,
I am,

Yours,

Harold E. Varmus, M.D.

P.S. Mike sends his regards.

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